Current Biology, Vol. 12, 1413–1418, August 20, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)01069-2

Rho-Kinase and Myosin-II Control Phagocytic Cup Formation during CR, but Not FcγR, Phagocytosis

Isabel M. Olazabal,[1] Emmanuelle Caron,[2,5] Robin C. May,[1,6] Kerstin Schilling,[3] David A. Knecht,[1,7] and Laura M. Machesky[1,4]

[1]School of Biosciences
University of Birmingham
Edgbaston
Birmingham B15 2TT
[2]MRC-LMCB
Laboratory for Molecular Cell Biology
and MRC Cell Biology Unit
University College London
Gower Street
London WC1E 6BT
United Kingdom
[3]Department of Cell Biology
GBF (National Research Centre for Biotechnology)
Mascheroderweg 1
38124 Braunschweig
Germany

Summary

Phagocytosis through Fcγ receptor (FcγR) or complement receptor 3 (CR) requires Arp2/3 complex-mediated actin polymerization, although each receptor uses a distinct signaling pathway [1]. Rac and Cdc42 are required for actin and Arp2/3 complex recruitment during FcγR phagocytosis, while Rho controls actin assembly at CR phagosomes [2, 3]. To better understand the role of Rho in CR phagocytosis, we tested the idea that a known target of Rho, Rho-kinase (ROK), might control phagocytic cup formation and/or engulfment of particles. Inhibitors of ROK (dominant-negative ROK and Y-27632) and of the downstream target of ROK, myosin-II (ML7, BDM, and dominant-negative myosin-II), were used to test this idea. We found that inhibition of the Rho → ROK → myosin-II pathway caused a decreased accumulation of Arp2/3 complex and F-actin around bound particles, which led to a reduction in CR-mediated phagocytic engulfment. FcγR-mediated phagocytosis, in contrast, was independent of Rho or ROK activity and was only dependent on myosin-II for particle internalization, not for actin cup formation. While myosins have been previously implicated in FcγR phagocytosis [4–6], to our knowledge, this is the first demonstration of a role for myosin-II in CR phagocytosis.

[4]Correspondence: l.m.machesky@bham.ac.uk
[5]Present address: Centre for Molecular Microbiology and Infection, Imperial College for Science, Technology and Medicine, The Flowers Building, Armstrong Road, London SW7 2AZ, United Kingdom.
[6]Present address: Hubrecht Laboratory, Uppsalalaan 8, 3584CT Utrecht, The Netherlands.
[7]Present address: Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269.

Results and Discussion

ROK Activity Is Required for CR-Mediated, but not FcγR-Mediated, Phagocytosis

Rho activity is specifically required during CR-mediated phagocytosis [2]; therefore, we analyzed whether Rho-kinase (ROK), a Rho downstream target [7], played a role in particle engulfment. Cells expressing ROK-K (an active construct used as a control [8]), ROK-KD (a catalytically dead mutant harboring a mutation in the ATP binding site and thought to act as a dominant negative [8]), and RhoA-N19 (dominant-negative Rho [2]) showed similar particle binding compared to control dextran-injected cells (data not shown, the Experimental Procedures are explained in the legend of Table 1). Cells expressing ROK-K and control cells showed a similar efficiency of internalization (Table 1). However, phagocytosis was markedly reduced in cells expressing RhoA-N19, as has been shown before [2], and in cells expressing ROK-KD (Table 1). We also used Y-27632, which is an inhibitor of the kinase activity of ROK, although it can also inhibit PRK2 [9, 10]. Cells treated with Y-27632 also showed reduced efficiency of internalization (Table 1), although neither particle binding (Figure S1A) nor overall actin morphology were affected (Figure 1Aa and data not shown). Together, these data indicate that ROK activity plays an important role in CR-mediated phagocytosis.

We analyzed whether ROK was also involved in FcγR-mediated phagocytosis in J774.A1 cells. Inhibition of ROK, either by expression of ROK-KD or Y-27632 treatment, caused no difference in the general actin morphology (Figure 1Ba and data not shown), particle binding (data not shown), or internalization compared to untreated cells (Table 1). Thus, the requirement for ROK activity seems to be specific to CR-mediated phagocytosis.

Myosin-II Is Recruited to CR Phagosomes during Phagocytosis

The requirement for ROK in CR phagocytosis suggests that its downstream target, myosin-II [7, 11], may also be involved. Two nonmuscle myosin-II isoforms are expressed in mammalian cells [12, 13]; however, blood cells only express myosin-IIA (Figure S2 in the Supplementary Material available with this article online and [14]). Myosin-IIA localization was observed on both CR and FcγR phagosomes in J774.A1 cells (Figures 2 and S1B), and its recruitment to CR phagosomes was studied at various stages of phagocytosis. "Stages" 0–4 of CR phagocytosis were arbitrarily defined, as explained in the figure legend of Figure 2. Myosin-IIA localization at the phagosomes was most apparent at stages 1–3, i.e., when the particles are still partially external or are just recently engulfed by the cell and, interestingly, when actin and Arp2/3 complex are associated with CR phagosomes [3] (Figure 2).

Table 1. Efficiency of Internalization in CR or FcγR Phagocytosis

CR Internalization (Percentage of Control)		
	J774.A1	Cos-7
Control	100	100
Myc-ROK-K	135 ± 28	110 ± 22
	n = 3	n = 3
Myc-ROK-KD	27 ± 14**	41 ± 29**
	n = 3	n = 3
Y-27632	61 ± 14**	60 ± 20
	n = 9	n = 2
ML7	61 ± 9**	30 ± 6
	n = 9	n = 2
BDM	42 ± 18**	45 ± 2
	n = 9	n = 2
GFP-Myosin-IIA	127 ± 6**	100 ± 17
	n = 3	n = 4
GFP-ΔN592	69 ± 15**	65 ± 4**
	n = 3	n = 4
FcγR Internalization (Percentage of Control)		
	J774.A1	Cos-7
Control	100	100
Myc-ROK-K	130 ± 6	n.d.
	n = 2	
Myc-ROK-KD	111 ± 40	n.d.
	n = 2	
Y-27632	118 ± 20	n.d.
	n = 3	
ML7	60 ± 17**	n.d.
	n = 6	
BDM	[4]	n.d.
GFP-Myosin-IIA	n.d.	100 ± 31
		n = 5
GFP-ΔN592	n.d.	69 ± 28**
		n = 5

J774.A1 or Cos-7 cells were transferred to HEPES-containing serum-free medium (SFM-H) for 3 hr prior to the phagocytosis assay. After microinjection, transfection, or drug treatment, opsonized particles were resuspended in SFM-H and were added to the cells at 37°C for 15 min. For CR-mediated phagocytosis, J774.A1 cells were first incubated with 150 ng/ml phorbol 12-myristate 13-acetate (PMA) for 15 min to induce CR activation [29]. Particles were stained as described previously [2, 3]. Particle binding was quantified by counting ten fields of phagocytic cells in duplicate for each condition in each experiment and did not vary compared with the respective controls (see Figure S1 in the Supplementary Material for titration assays). Internalization of particles was counted as the number of internalized particles per 100 bound particles. The statistical significance of these data was tested using a paired Student's t test with a goodness-of-fit of $p < 0.05$ (*) or $p < 0.01$ (**). The results are expressed as the percentage of internalization in cells under different conditions compared with internalization of their respective controls (percentage of control) and are the mean of "n" experiments ± the standard deviation.

Myosin-II Plays a Role in Particle Internalization during Both CR- and FcγR-Mediated Phagocytosis

Myosin-II activity controls FcγR-mediated phagocytosis [4, 5]. To determine whether myosin-II was also required for CR phagocytosis, we made use of two chemical inhibitors: ML7, which inhibits myosin light chain kinase (MLCK) [15], and BDM, previously described to block the ATPase activity of myosin-II [16]. None of the inhibitors affected the overall actin morphology of the cells at the concentrations used (Figures 1Aa and 1Ba and data not shown), nor did they affect particle binding (Figures S1B–S1C). However, the efficiency of CR-mediated phagocytosis in treated cells was reduced compared with that in untreated cells (Table 1). ML7 also reduced the efficiency of internalization for FcγR phagocytosis (Table 1), which is consistent with previous data for BDM [4, 5]. These results confirm the role of myosin-II during FcγR-mediated phagocytosis. Importantly, our data now suggest a role for myosin-II activity in the CR-dependent phagocytic pathway.

Since these inhibitors may have a broader specificity than previously recognized [17], we also used a dominant-negative fragment of the nonmuscle myosin-IIA heavy chain. This construct, lacking amino acids 1–591 (ΔN592), has lost its abilities to bind ATP and actin (even though actin binding sequences are still present) but is still able to assemble myosin filaments, thereby inhibiting contractility [18]. GFP-myosin-IIA or GFP-ΔN592 were expressed in Cos-7 cells. We assayed phagocytosis at the time point at which the expression levels of GFP-ΔN592 were sufficient to disrupt stress fibers, but cell rounding had not yet taken place, i.e., 20–24 hr [18]. None of the myosin-II constructs affected particle binding (data not shown). However, GFP-ΔN592-expressing cells showed a reduced ability to internalize through either the FcγR or the CR when compared to control cells or GFP-myosin-IIA-expressing cells (Table 1). The inhibitory effect of GFP-ΔN592 on CR-mediated phagocytosis was also observed in microinjected J774.A1 macrophages (Table 1). In contrast to ROK-KD and BDM, which appear to block CR phagocytosis, GFP-ΔN592, ML7, and Y-27632 all seem to delay the CR phagocytic process (time-course experiments in Figures S2A–S2C and S3 and data not shown). This could suggest that either other pathways downstream of Rho might play a role in CR phagocytosis or, more likely, that the inhibitors are not completely effective in blocking protein function. Nevertheless, our data indicate that myosin-IIA is involved in particle internalization during both CR- and FcγR-mediated phagocytosis.

Myosin-II Is Required for Actin Cup Assembly during CR, but Not FcγR, Phagocytosis

Since both CR and FcγR phagocytosis appear to use myosin-IIA activity for particle engulfment, we examined whether ROK and MLCK directed myosin-IIA recruitment to the phagosome. Therefore, we quantified the percentage of phagocytic cups that were positive for myosin-IIA in control cells and in cells treated with ROK and MLCK inhibitors. ML7 prevented the recruitment of myosin-IIA to both CR and FcγR phagosomes, while Y-27632 inhibited myosin-IIA recruitment to CR phagosomes (Table 2). Y-27632 had no effect on FcγR uptake (Table 1); therefore, we did not analyze its involvement in FcγR recruitment in any more detail.

Since Rho activity is required for the recruitment of Arp2/3 complex and actin filaments to the phagocytic cup during CR phagocytosis [3], we hypothesized that ROK and myosin-II might be part of this pathway. We observed that cells treated with Y-27632, ML7, or BDM showed a significantly reduced number of particles ac-

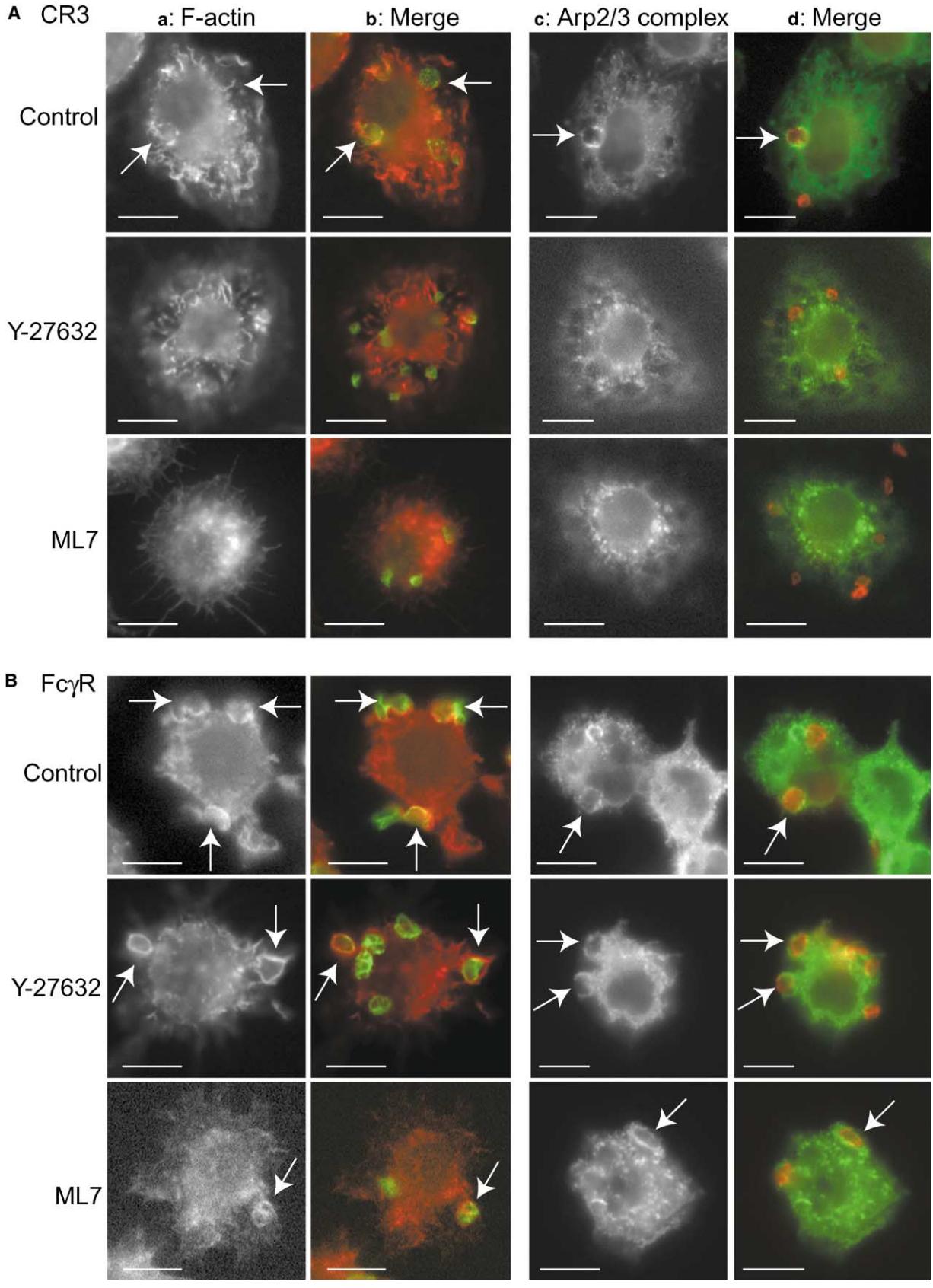

Figure 1. F-Actin and Arp2/3 Complex Recruitment to Phagosomes in Cells Treated with ROK and MLCK Inhibitors

(A and B) Macrophages were incubated with Y-27632 or ML7 prior to the (A) CR or (B) FcγR phagocytic assay. Cells were stained for (a) F-actin and (b) RBC (merged in [b], F-actin in red, and RBC in green) or (c) Arp2/3 complex and (d) RBC (merged in [d], Arp2/3 complex in green, and RBC in red). Arrows indicate the recruitment of F-actin or Arp2/3 complex to phagosomes. (A) Note that, in CR phagocytosis, there is a reduced number of internalized RBC and a reduced recruitment of actin and Arp2/3 complex to phagosomes, in cells treated with Y-27632 or ML7. (B) In contrast, during FcγR phagocytosis, treatment with ML7 cells only reduced internalization and had no effect on the recruitment of actin or Arp2/3 complex. The scale bars represent 10 μm.

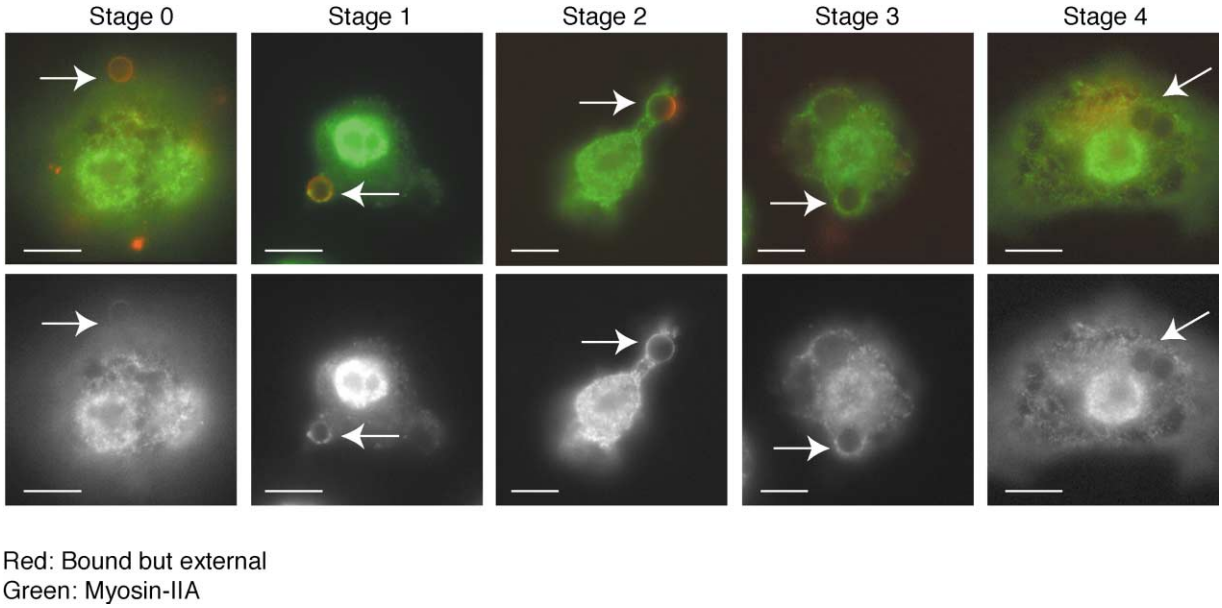

Figure 2. Localization of Myosin-IIA to the CR Phagosome during the Different Stages of Internalization

CR phagocytosis was assayed, and macrophages were stained for external beads (prior to permeabilization, red) and myosin-IIA (green, bottom panel). Merged images are shown in the top panel. Stages 0–4 are defined as follows: (0) particle is bound, but no cup is visible; (1–2) a fraction of the particle is still outside the cell; (3–4) the particle is inside the cell, near the periphery (indicating possible recent entry) (3), or near the center of the cell (4). Myosin-IIA is localized to the cup in stages 1–3. Stage 0 is typically observed at the beginning of the experiment; by 5–20 min, stages 1–3 were predominant, while stage 4 was observed almost exclusively in cells left for 60 min or more. A bead is 3 μm in diameter.

cumulating Arp2/3 complex or F-actin at phagocytic cups during CR phagocytosis (Figure 1A and quantified in Table 2). On the contrary, ML7 did not affect the recruitment of these proteins to FcγR-dependent phagosomes (Figure 1B and Table 2), which is consistent with previous data for BDM [4].

Our observations, combined with those of other labs, are summarized in Figure 3. ROK and MLCK contribute to the localization of myosin-IIA, actin assembly, and particle engulfment during CR phagocytosis. In contrast, ROK does not appear to be involved in FcγR phagocytosis. In the FcγR pathway, inhibition of MLCK caused a significant reduction of both myosin-IIA localization and particle uptake, while it did not affect actin or Arp2/3 complex recruitment, suggesting a role for MLCK and myosin-IIA in later steps of phagosome formation, after the actin cup has assembled, in agreement with previous findings [4, 5]. Our data implicate MLCK and/or the regulation of myosin-II activity during actin assembly at CR phagosomes and possibly during the closure of FcγR phagosomes. This contrasts with published observations in which phagocytosis of IgG-opsonized yeast was not affected by injection of antibodies against MLCK [19]. However, this might be explained by differences in the modes of action of MLCK antibodies and inhibitors and/or differences in the nature of the phagocytic targets that were used. Nonopsonized yeasts can be recognized by other phagocytic receptors [20] that may induce different signaling pathways.

The requirement for myosin-II in CR-induced actin assembly is particularly intriguing, and we do not yet understand its molecular basis. There is no striking effect of myosin-II inhibition on the general localization of Arp2/3 complex in cells expressing the myosin-II mutant GFP-ΔN592 (see Figure S4A, where Arp2/3 complex and F-actin are typically enriched in the lamellipodia). However, myosin-IIA does colocalize with Arp2/3 complex and F-actin at early (stages 1–3) CR phagosomes (Figure S4B). Thus, CR-mediated phagocytosis specifically uses myosin-II to assist in the assembly of actin cups around particles. We do not think it likely that myosin-II is generally connected with Arp2/3 complex-mediated actin assembly, but there may be other specific cellular

Table 2. Efficiency of Myosin-II, F-Actin, and Arp2/3 Complex Recruitment to CR and FcγR Phagosomes

J774.A1			
CR Recruitment (Percentage of Control)			
	Myosin-IIA	F-Actin	Arp2/3 Complex
Control	100	100	100
Y-27632	49 ± 10*	56 ± 24**	56 ± 9*
	n = 4	n = 3	n = 3
ML7	39 ± 11*	51 ± 14**	38 ± 17**
	n = 4	n = 6	n = 4
BDM	[4]	34 ± 22**	23 ± 24**
		n = 6	n = 5
FcγR Recruitment (Percentage of Control)			
	Myosin-IIA	F-Actin	Arp2/3 Complex
Control	100	100	100
ML7	40 ± 12*	122 ± 33	93 ± 7
	n = 3	n = 4	n = 4
BDM	n.d.	[4]	[4]

Data are expressed as in Table 1.

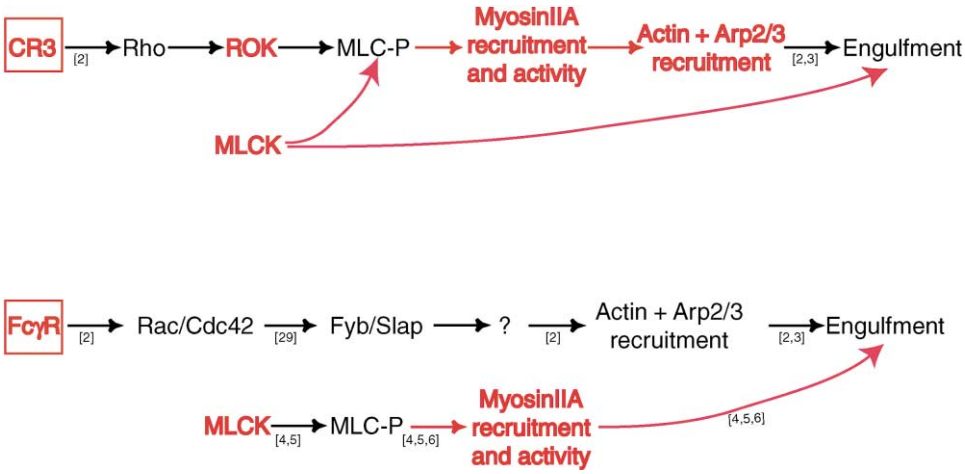

Figure 3. Schematic Representation of CR and FcγR Phagocytic Pathways

ROK and MLCK are important for myosin-IIA localization and activity leading to actin assembly and particle engulfment in CR phagocytosis. In contrast, during FcγR-mediated phagocytosis, actin assembly is independent of Rho, ROK, MLCK, and myosin-IIA. Rac and Cdc42 mediate the actin assembly at FcγR phagosomes possibly through the Fyb/SLAP protein, which appears to interact with Arp2/3 complex via WASp-family proteins [28]. Our observations are shown in red. The numbers indicated on each pathway refer to published sources of the data (see references).

processes that, like CR phagosomes, utilize this connection.

We propose two models for how myosin-IIA might assist in actin assembly at CR phagosomes. In the first scenario, myosin-IIA directly recruits Arp2/3 complex or uses intermediate binding protein(s) other than actin. Therefore, inhibition of myosin-IIA would block Arp2/3 complex-mediated actin filament formation. A direct connection between myosin-I and Arp2/3 complex has already been established in which myosin-I may transport Arp2/3 complex to the membrane [21–23]. However, since nonmuscle myosin-II from mammalian cells lacks the specific regions required for a similar type of interaction, and there is no known mechanism for a similar directed movement of myosin-II, this model seems unlikely. The second possibility is that myosin-IIA is required for initial actin assembly in the phagocytic cup. This could be mediated by the actin binding site in the motor domain, since the S1 subfragment of myosin has been shown to induce actin polymerization in vitro [24–26]. Our second model is similar to previous models in which myosin-II arrives at the early stages of cleavage furrow formation and possibly promotes the accumulation of actin filaments in this area [27]. Once sufficient actin has accumulated at the phagosome, Arp2/3 complex could presumably bind to the filaments and induce the formation of dendritic networks.

Supplementary Material

Supplementary Material including the Experimental Procedures and four Supplementary Figures is available at http://images.cellpress.com/supmat/supmatin.htm. Figures S1 and S3 show time courses and titrations of the different drug treatments and myosin constructs during phagocytosis, Figure S2 shows the localization of myosin isoforms, and Figure S4 shows the specificity of the connection between Arp2/3 complex and myosin-II.

Acknowledgments

We thank R. Adelstein and Q. Wei for providing the antibodies against myosin-IIA and -IIB and the constructs containing myosin-IIA and its mutant; M. Peckham, J. Kendrick-Jones, and R. Insall for helpful advice; and B. Behrendt and M. Tchibalina for critical reading of the manuscript. L.M.M. is supported by a Medical Research Council Senior Research Fellowship. I.M.O. is supported by a Human Frontiers Society Program Organization (HFSPO) grant to L.M.M. E.C. is supported by the Wellcome Trust. K.S. is supported by an HFSPO grant to Juergen Wehland. D.A.K. is supported by a grant from the National Institutes of Health (GM40599).

Received: October 23, 2001
Revised: May 20, 2002
Accepted: July 2, 2002
Published: August 20, 2002

References

1. Kaplan, G. (1977). Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages. Scand. J. Immunol. *6*, 797–807.
2. Caron, E., and Hall, A. (1998). Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. Science *282*, 1717–1721.
3. May, R.C., Caron, E., Hall, A., and Machesky, L.M. (2000). Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3. Nat. Cell Biol. *2*, 246–248.
4. Swanson, J.A., Johnson, M.T., Beningo, K., Post, P., Mooseker, M., and Araki, N. (1999). A contractile activity that closes phagosomes in macrophages. J. Cell Sci. *112*, 307–316.
5. Mansfield, P.J., Shayman, J.A., and Boxer, L.A. (2000). Regulation of polymorphonuclear leukocyte phagocytosis by myosin light chain kinase after activation of mitogen-activated protein kinase. Blood *95*, 2407–2412.
6. Diakonova, M., Bokoch, G., and Swanson, J.A. (2002). Dynamics of cytoskeletal proteins during Fcγ receptor-mediated phagocytosis in macrophages. Mol. Biol. Cell *13*, 402–411.
7. Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y., and Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). J. Biol. Chem. *271*, 20246–20249.
8. Amano, M., Chihara, K., Kimura, K., Fukata, Y., Nakamura, N., Matsuura, Y., and Kaibuchi, K. (1997). Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. Science *275*, 1308–1311.
9. Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M.,

et al. (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature *389*, 990–994.
10. Davies, S.P., Reddy, H., Caivano, M., and Cohen, P. (2000). Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem. J. *351*, 95–105.
11. Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., et al. (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science *273*, 245–248.
12. Katsuragawa, Y., Yanagisawa, M., Inoue, A., and Masaki, T. (1989). Two distinct nonmuscle myosin-heavy-chain mRNAs are differentially expressed in various chicken tissues. Identification of a novel gene family of vertebrate non-sarcomeric myosin heavy chains. Eur. J. Biochem. *184*, 611–616.
13. Kawamoto, S., and Adelstein, R.S. (1991). Chicken nonmuscle myosin heavy chains: differential expression of two mRNAs and evidence for two different polypeptides. J. Cell Biol. *112*, 915–924.
14. Maupin, P., Phillips, C.L., Adelstein, R.S., and Pollard, T.D. (1994). Differential localization of myosin-II isozymes in human cultured cells and blood cells. J. Cell Sci. *107*, 3077–3090.
15. Saitoh, M., Ishikawa, T., Matsushima, S., Naka, M., and Hidaka, H. (1987). Selective inhibition of catalytic activity of smooth muscle myosin light chain kinase. J. Biol. Chem. *262*, 7796–7801.
16. Mitchison, T.J., and Cramer, L.P. (1996). Actin-based cell motility and cell locomotion. Cell *84*, 371–379.
17. Cheung, A., Dantzig, J.A., Hollingworth, S., Baylor, S.M., Goldman, Y.E., Mitchison, T.J., and Straight, A.F. (2002). A small-molecule inhibitor of skeletal muscle myosin II. Nat. Cell Biol. *4*, 83–88.
18. Wei, Q., and Adelstein, R.S. (2000). Conditional expression of a truncated fragment of nonmuscle myosin II-A alters cell shape but not cytokinesis in HeLa cells. Mol. Biol. Cell *11*, 3617–3627.
19. de Lanerolle, P., Gorgas, G., Li, X., and Schluns, K. (1993). Myosin light chain phosphorylation does not increase during yeast phagocytosis by macrophages. J. Biol. Chem. *268*, 16883–16886.
20. Brown, G.D., and Gordon, S. (2001). Immune recognition. A new receptor for beta-glucans. Nature *413*, 36–37.
21. Lee, W.L., Bezanilla, M., and Pollard, T.D. (2000). Fission yeast myosin-I, Myo1p, stimulates actin assembly by Arp2/3 complex and shares functions with WASp. J. Cell Biol. *151*, 789–800.
22. Evangelista, M., Klebl, B.M., Tong, A.H., Webb, B.A., Leeuw, T., Leberer, E., Whiteway, M., Thomas, D.Y., and Boone, C. (2000). A role for myosin-I in actin assembly through interactions with Vrp1p, Bee1p, and the Arp2/3 complex. J. Cell Biol. *148*, 353–362.
23. Jung, G., Remmert, K., Wu, X., Volosky, J.M., and Hammer, J.A., 3rd. (2001). The *Dictyostelium* CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. J. Cell Biol. *153*, 1479–1497.
24. Miller, L., Phillips, M., and Reisler, E. (1988). Polymerization of G-actin by myosin subfragment 1. J. Biol. Chem. *263*, 1996–2002.
25. Eto, M., Morita, F., Nishi, N., Tokura, S., Ito, T., and Takahashi, K. (1991). Actin polymerization promoted by a heptapeptide, an analog of the actin-binding S site on myosin head. J. Biol. Chem. *266*, 18233–18236.
26. Carlier, M.F., Didry, D., Erk, I., Lepault, J., and Pantaloni, D. (1994). Myosin subfragment-1-induced polymerization of G-actin. Formation of partially decorated filaments at high actin-S1 ratios. J. Biol. Chem. *269*, 3829–3837.
27. Li, F., Wang, X., Bunger, P.C., and Gerdes, A.M. (1997). Formation of binucleated cardiac myocytes in rat heart: I. Role of actin-myosin contractile ring. J. Mol. Cell. Cardiol. *29*, 1541–1551.
28. Coppolino, M.G., Krause, M., Hagendorff, P., Monner, D.A., Trimble, W., Grinstein, S., Wehland, J., and Sechi, A.S. (2001). Evidence for a molecular complex consisting of Fyb/SLAP, SLP-76, Nck, VASP and WASP that links the actin cytoskeleton to Fcγ receptor signalling during phagocytosis. J. Cell Sci. *114*, 4307–4318.
29. Wright, S.D., Craigmyle, L.S., and Silverstein, S.C. (1983). Fibronectin and serum amyloid P component stimulate C3b- and C3bi-mediated phagocytosis in cultured human monocytes. J. Exp. Med. *158*, 1338–1343.